[Jpn. J. Parasitol., Vol. 38, No. 5, 280—284, October, 1989]

Prevalence of *Toxocara* Species Eggs in the Sandpits of Public Parks in Hyogo Prefecture, Japan

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(Accepted for publication; July 13, 1989)

Abstract

Toxocara species eggs were found in 95 of 227 (41.9%) sand samples collected from public parks in urban, residential and rural areas in Hyogo Prefecture, Japan. The contamination rate in urban areas was significantly higher than in the other two areas. The highest number of eggs recovered from 20 g of sand was 128, which was equivalent to 16 eggs/g in the original sand sample, based on a recovery efficiency of 40% using our method. Of the eggs recovered, 85% were fully embryonated and some embryonated eggs examined were infective to mice. The contamination ratio of *T. canis* to *T. cati* was 1 : 3, as determined by scanning electron microscope observations and the measurement of the egg size. More control of pollution in sandpits in public parks is needed.

Key words: *Toxocara* species eggs, sandpits, parks

Introduction

Toxocara canis and *T. cati,* the common intestinal helminth parasites of dogs and cats, are cosmopolitan species and are known to infect humans resulting in a condition known as visceral larva migrans (VLM). VLM in humans is most frequently caused by *T. canis* and is most commonly reported in young children. Children are considered to be particularly at risk of infection because of their pica habits, close association with pets and playing environments, e.g., public parks. *T. cati* may also be potentially significant, although definitive evidence of its role in causing VLM is lacking.

Studies on the prevalence of *Toxocara* spp. eggs in public places have been made in many parts of the world. In the United Kingdom, 11 to 66% of soil samples from public parks were shown to contain *Toxocara* spp. eggs (Borg and Woodruff, 1973; Quinn *et al.*, 1980; Snow *et al.*, 1987). In the United States, soil samples were collected from gardens, backyards and/or highway rest areas, which revealed that 11 to 22% were contaminated (Dada and Lindquist, 1979; Childs, 1985). In addition to these, similar studies have been made in Australia (Collins and Moore, 1982)., Nigeria (Chiejina and Ekwe, 1986) and Germany (Duwel, 1984). In Japan, 1.5 to 12.0% of the dog stool samples collected in parks and roads were positive for *T. canis* eggs (Kondo, 1989).

The purpose of this study was to establish the prevalence of *Toxocara* spp. eggs and to investigate the distribution ratio of both species of eggs in the sandpits of public parks in Hyogo Prefecture.

Materials and Methods

Study areas

The study was carried out over a period of 7 months from September 1988 through March 1989 in Hyogo Prefecture, Japan. Sand samples were collected from public parks in three different areas: urban areas crowded with houses, new residential areas in the suburbs and rural areas.

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Recovery of sand

Sand was taken from the sandpits of public parks using a 6 cm diameter plastic pipe, with the edge serrated to aid cutting the soil, to a depth of approximately 3 cm. A sample of 100—150 g sand per four square metres was taken, which corresponded to one to sixteen samples per sandpit. The sand samples in the same park were thoroughly mixed and stored in sealed and labelled polythene bags. Samples were taken to the laboratory, dried overnight at room temperature, and then sifted through sieves of 1 mm mesh to remove stones and grass. When the samples were collected, the presence of animal feces in the sandpits was also recorded.

Laboratory procedures

Twenty grams of samples were weighed and mixed with 50 ml of 0.05% Tween 80 solution. The whole mixture was vigorously stirred and sieved through 170 μm. The sample was then transferred to a 50 ml round bottomed centrifuge tube and centrifuged at 200 $\times$ g for 5 min. The supernatant was sucked off. The precipitate was resuspended in 5 to 6 ml of water, transferred to a 10 ml centrifuge tube and was again centrifuged under the same conditions. After the centrifugation, the supernatant was discarded and a sucrose solution of specific gravity 1.200 was then added to 1 cm from the top. The cork was placed and the tube shaken thoroughly using a LAB-Mixer (Iuchi Co.) for more than 30 seconds. The tube was again centrifuged at 550 $\times$ g for 15 min. After the centrifugation, the tube was then filled to the brim with sucrose solution so that only a small air bubble was formed when a coverslip (18 mm $\times$ 18 mm) was placed on the tube. The tube was allowed to stand for 2 hr and the coverslip on the tube was removed onto a microscope slide. For the second examination to detect the remaining eggs, the inside of the tube was scratched 50 times using a small fine metal wire. A coverslip was superimposed on the tube, and left overnight.

Slides were examined microscopically for the presence of eggs, and if either of the two samples contained *Toxocara* spp. eggs, the examination was considered positive. The total number of eggs recovered from two specimens was counted and classified based on the developing stages into 3 types, namely, mono-cell, 2 to pre-embryonated cell and embryonated cell. The motility of embryonated eggs was judged according to the movement and shape of larvae.

Other tests

To differentiate *T. canis* from *T. cati,* both species of eggs were recovered from the uteri of adult worms and cultured *in vitro*. The major and minor axes of both species of eggs were measured every 3 days of cultivation. Using artificially seeded sand samples with cultured *T. canis* eggs, recovery rate of the eggs was studied. Some of the eggs obtained from parks were observed using a scanning electron microscope (SEM; JEOL, JSM-330A) by ordinary and easy methods of preparation. In the easy method, a drop of egg suspension on the coverslip was dried up in a desiccator overnight and observed by means of SEM without any fixation and dehydration of the specimens. Furthermore, experiments were carried out to determine whether these eggs could produce an active infection in mice.

Results

Of the 227 samples collected, 41.9% were found to be contaminated with *Toxocara* spp. eggs. Contamination rates were 68.8, 18.4 and 13.0% in urban, residential and suburb areas, respectively. The value in the urban areas was significantly higher than that in residential ($P<0.05$) and suburban areas ($P<0.001$). The egg count for the positive sand samples varied from 1 to 128 eggs/20 g of sand with a mean of 4.5. Animal feces were found in 106 of 227 (46.7%) parks sampled (Table 1).

Table 2 shows the total number of eggs recovered in the three areas and their stage of development. A total of 1,007 eggs were recovered and 864 (85.8%) of them were fully embryonated. There were no significant differences ($P>0.05$) in embryonation rate among the three areas examined. Three-hundred and thirty-seven embryonated eggs recovered were further examined, which revealed that 95.7% of the larvae were motile. Some of the embryonated

Table 1. Prevalence of *Toxocara* species eggs in sandpits of public parks

Areas	No. parks examined	No. positive parks (%)		No. eggs recovered/20 g	
		Toxocara	Feces	Min.-Max.	Mean
Urban	109	75 (68.8)	68 (62.4)	1—128	19.5
Residential	87	16 (18.4)	34 (39.1)	1— 4	1.7
Rural	31	4 (13.0)	4 (12.9)	1— 1	1.0
Total	227	95 (41.9)	106 (46.7)	1—128	4.5

Table 2. Number of *Toxocara* species eggs recovered and their stage of development

Areas	No. eggs recovered	No. eggs developed to: (%)		
		1 cell	2～Pre-emb.*	Emb.†
Urban	962	8 (0.8)	131 (13.6)	823 (85.6)
Residential	40	1 (2.5)	3 (7.5)	36 (90.0)
Rural	5	0 (0.0)	0 (0.0)	5 (100.0)
Total	1,007	9 (0.9)	134 (13.3)	864 (85.8)

* Pre-embryonated egg stage.
† Embryonated egg stage.

eggs were administered to mice and 48 hr after inoculation, second-stage larvae were recovered from livers, indicating that the eggs were infective (data not shown).

The major and minor axes of the eggs (n = 158) of *T. canis* and *T. cati* recovered from the uteri were measured at different stages of development. As a result, the major and minor axes of *T. canis* were 76.3—93.5 μm and 61.2—78.0 μm and those of *T. cati* were 67.3—82.5 μm and 53.4—68.0 μm, respectively (Fig. 1). There were no significant differences in the size of the eggs irrespective of their developmental stages in each species. The size of these eggs was compared with that of the eggs (n = 165) recovered from parks (Fig. 1). Of the eggs recovered from parks, 6.1% were significantly larger in both major and minor axes than those of *T. cati* and were identified as *T. canis* (○). In the same manner, 19.4% were identified as *T. cati* (●). However, most of the eggs, 75.4%, remained unidentified (△).

Scanning electron microscopic observations using the ordinary preparation method clearly distinguished between *T. canis* and *T. cati* (Fig. 2 a, b). The easy preparation method was also useful, although a majority of the eggs were shrunken (Fig. 2 c, d). Fifty-seven eggs recovered from sand samples were prepared by the easy method and the observation revealed that 23.5% of them were *T. canis* and the remaining 76.5% were *T. cati*. This ratio of *T. canis* and *T. cati* was similar to the result in Fig. 1. Other than *Toxocara* spp. eggs, *Trichuris vulpis* (0.1%) and *Capillaria* sp. (0.1%) were found but no protozoal oocysts were observed.

Discussion

Based on our data obtained from the same study area, the prevalence rates of *T. canis* among puppies and adult dogs were 67.0% and 18.4%, respectively (Uga *et al.*, 1982). Similarly, the prevalence rate of *T. cati* among cats was 22.0% (Uga *et al.*, 1983). These parasites were distributed in all 3 areas with no regional differences in prevalence rate. However, sand samples obtained from urban areas were more frequently contaminated with *Toxocara* spp. eggs than those from residential and rural areas. This

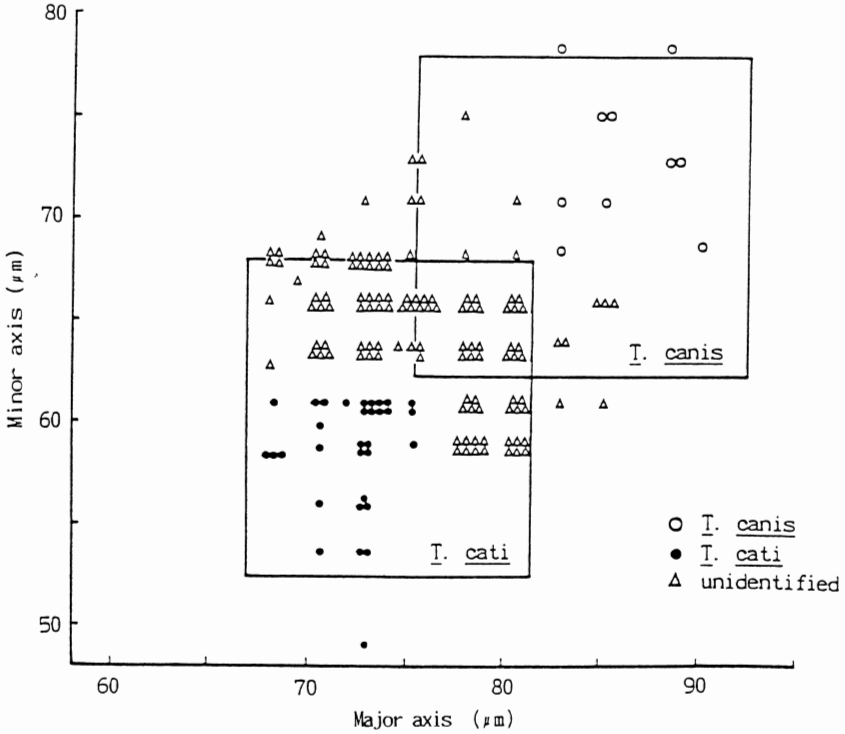

Fig. 1. Distribution patterns of eggs of *Toxocara canis* and *T. cati* recovered from sandpits of public parks. Based on the egg size, a part of samples (○) was identified as *T. canis* and another part (●) as *T. cati* with the other part (△) unidentified.

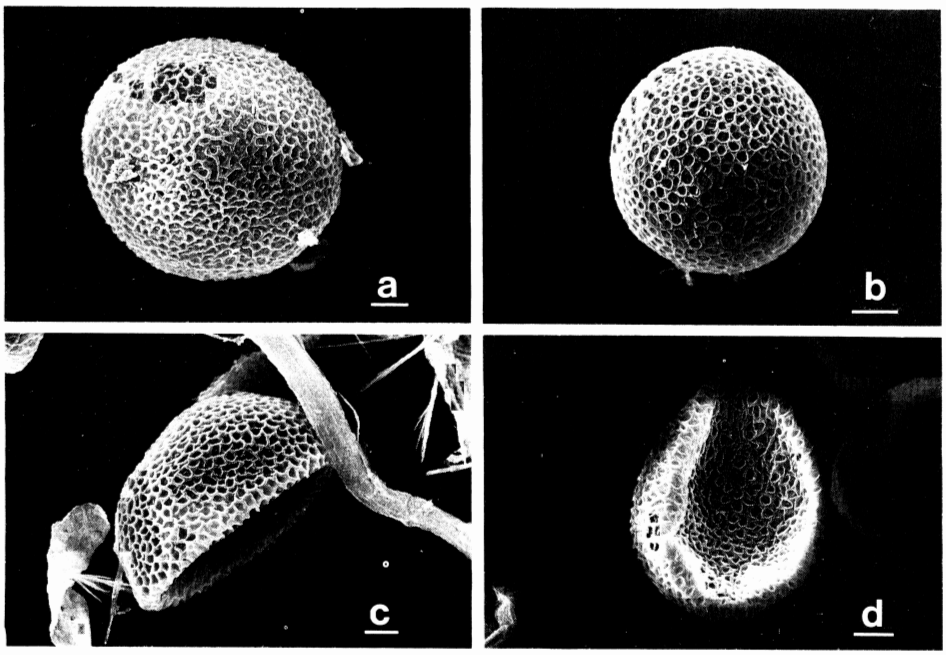

Fig. 2. Scanning electron microscopic observations of eggs of both *Toxocara canis* (a, c) and *T. cati* (b, d) using the ordinary preparation method (a, b) and the easy method (c, d). Bar = 10μm.

difference may be attributed to environmental conditions. In urban areas with the highest prevalence, almost all the ground except parks were paved, so that the dogs and cats might swarm around sandpits for defecation. This contrasts with the rural area showing the lowest prevalence, where the dogs and cats have more room for defecation. These are supported by the fact that the fecal contamination rate in the sandpits was significantly higher ($P < 0.001$) in urban areas than the other two areas. The highest number of eggs recovered from 20 g of sand was 128. Its density was estimated to be 16 eggs/g based on the recovery efficiency (40%).

To assess the soil contamination with *Toxocara* spp. eggs, a simple, accurate and reproducible egg recovery method is required. Preliminary studies using artificially seeded sand samples have demonstrated a mean recovery efficiency of 40%, which was lower than that of Dada (1979), Quinn *et al.* (1980) and Collins and Moore (1982). Zinc sulphate, with a specific gravity of 1.180 to 1.320, was widely used as a flotation solution, however, we used a sucrose solution (specific gravity 1.200) for economy and safety (zinc sulfate is toxic). A lower recovery efficiency might be due to the difference in flotation solution.

It is reported that VLM in humans is more frequently caused by *T. canis* than *T. cati* (Elliot *et al.*, 1985). Because of the close morphological similarity between the eggs of *T. canis* and *T. cati*, little attempt has been made to differentiate between them in surveys reported so far (Duwel, 1984; Childs, 1985; Snow *et al.*, 1987). Duwel (1984) suspected that the eggs recovered from sand were probably *T. canis* since there are a large number of dogs in their study area, Frankfurt/Main. Snow *et al.* (1987) assumed that most of the eggs recovered from east London parks would be those of *T. canis* because of the difference in defecation habits of dogs and cats. Our study, however, revealed that the eggs of *T. canis* and *T. cati* were contained at an approximate ratio of 1:3.

Our result on the prevalence of *Toxocara* spp. eggs is consistent with the findings of other workers which show that the contamination with *Toxocara* spp. eggs is widespread in the soil and sand in public places (Dada and Lindquist, 1979; Duwel, 1984). It is apparent that sandpits in public parks in Hyogo Prefecture are commonly contaminated with animal feces and *Toxocara* spp. eggs are potentially infective to humans.

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